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LIPID CHANGES DURING DEVELOPMENT OF *BLASTOCLADIELLA EMERSONII*

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Key Word Index—*Blastocladieles emersonii*; aquatic fungus; zoospore encystment; neutral lipid; glycolipid; phospholipid; development; ^{14}C.

Abstract—The lipid composition of swimming spores, cysts and five hour germlings was established. Spores utilized triglycerides first, then phospholipids. Upon encystment all glycolipid components decreased, while in germlings the phospholipids, monoglycerides and sterol esters exhibited a marked increase.

INTRODUCTION

FUNGI accumulate lipids[1] in the form of globules that probably serve as reserves.[2] The zoospores and plants of the aquatic fungus *Blastocladiella emersonii* Cantino and Hyatt also contain lipid bodies.[3–5] Under conditions of starvation these zoospores utilize stored polysaccharides and lipid.[6] Recently the lipids have been identified quantitatively.[7] The present report documents changes in lipid classes and their individual components during different developmental stages of *B. emersonii*.

RESULTS

Radioactivity from NaOAc-[U-^{14}C] was incorporated into the lipids of synchronously produced zoospores (Fig. 1, 0 hr). Following fractionation into neutral lipids, glycolipids and phospholipids, the percentage of each class was determined by both dry weight and total ^{14}C incorporated (Table 1, 0 hr). Individual classes were separated by TLC (Fig. 2, 0 hr) and the major lipid components were identified[7] and quantified by measuring their total radioactivity (Fig. 2, 0 hr). The TG* and FS were the major neutral lipids, SE and MG being minor components with undectable label. The major glycolipid was DGDG, while the minor glycolipids were MGDG and PGDG. The phospholipid fraction contained two major peaks; one consisted of PC and LPC, the other contained PE and LPE. Minor phospholipids were PS and PI.

* Abbreviations used: TG = triglycerides; FS = free sterols; SE = sterol esters; MG-monoglycerides; MGDG, DGDG, PGDG = mono-, di- and polyglycosyldiglycerides, respectively; PC, LPC, PE, LPE, PS and PI = phosphatidylcholine, lysophosphatidylchloline, phosphatidylethanolamine, lysophosphatidylethanolamine, phosphatidylserine and phosphatidylinositol.

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These data were then compared with the results obtained from zoospores which had been swimming for 5 and 10 hr, from encysted spores, and from 5 hr germlings.

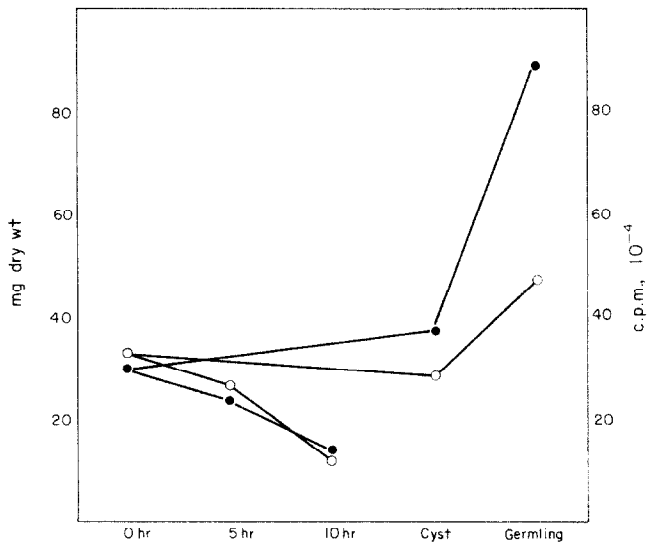

FIG. 1. CHANGES IN TOTAL (●——●) AND LABELLED (○——○) LIPID/10^{10} CELLS DURING SWIMMING AND DEVELOPMENT.
Lower curve, swimming spores; upper curve, encystment and growth.

Distribution of lipids in swimming zoospores

Zoospores were kept swimming in a non-nutrient buffered medium for either 5 or 10 hr. Total lipid was then extracted and separated into classes and individual components as before. Total lipid, whether measured by dry weight or ^{14}C incorporation, decreased as spores were starved (Fig. 1; 0, 5 and 10 hr). The per cent distributions for neutral lipids, glycolipids and phospholipids in the above were also compared (Table 1).

TABLE 1. THE PER-CENT COMPOSITION OF LIPID DURING SWIMMING AND DEVELOPMENT

Stage	Neutral lipid		Glycolipid		Phospholipid	
	Dry wt	cpm	Dry wt	cpm	Dry wt	cpm
0 hr	19·9	21·3	17·8	17·2	62·3	61·5
5 hr	15·9	11·6	18·6	15·1	65·5	73·3
10 hr	25·8	22·6	24·2	24·7	50·0	52·7
Cyst	19·2	21·0	9·5	8·4	71·3	70·6
Germling	19·9	14·8	8·5	12·0	71·6	73·2

The individual lipid components within each class were examined (Fig. 2). The greatest change in the neutral lipid was in the TG; they decreased 64% after 5 hr, then increased. Among the minor neutral lipids, SE and FS increased and decreased, respectively. Both complex glycolipids (PGDG) decreased after 5 and 10 hr, while the MGDG decreased initially but then increased. The major glycolipid, a DGDG, also increased after 10 hr. During the first 5 hr the major phospholipids changed slightly, but after 10 hr they decreased sharply. When compared to 0 hr spores, the peaks containing PE and LPE, and PC and

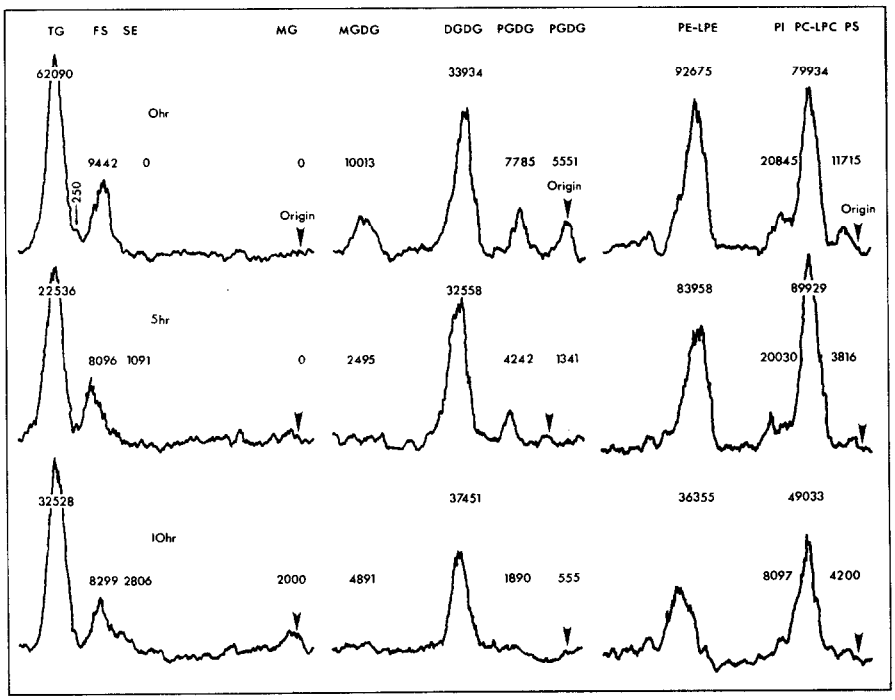

FIG. 2. CHANGES IN PROFILES OF LABELED LIPIDS IN ZOOSPORES DURING SWIMMING. For identity of peaks, see Abbreviations. Tabulated numbers are cpm incorporated in each peak/10^{10} spores. Full scale deflection was 100 cpm except where indicated (arrows).

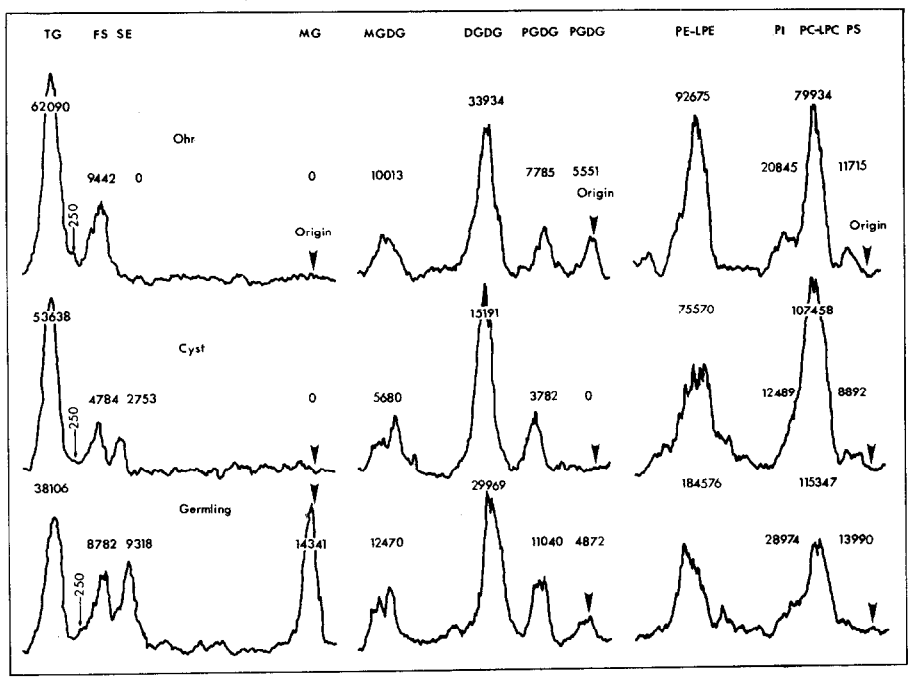

FIG. 3. CHANGES IN PROFILES OF LABELED LIPIDS IN ZOOSPORES, CYSTS AND GERMLINGS.

LPC, dropped by 61 and 39% respectively. PS and PI both decreased over the 10 hr period.

Distribution of lipid in encysted spores and in germlings

Lipid was extracted from zoospores which had been induced to encyst and from germlings which had been grown in a nutrient medium. As before, the lipid was separated first into classes and then into individual components. The sp. act., as expected, decreased since no additional ^{14}C had been added (Fig. 1; cysts and germlings). However, the per cent composition (Table 1) for each of the classes, as determined by total ^{14}C incorporated, was in reasonable agreement with that calculated from their respective dry weights. When 0 hr spores were compared to cysts and germlings (Table 1) it was found that the neutral lipids remained constant, the glycolipids decreased, and the phospholipids increased.

Changes occurring in the major lipid components can be followed in Fig. 3. With respect to neutral lipids, it is evident that the labeled TG decreased, while the SE increased. On the other hand, the FS dropped during encystment but then increased upon subsequent growth. One particularly conspicuous change in the neutral lipids of germlings was the appearance of the previously non-detectable MG. As for glycolipids, 55% of the DGDG and essentially all of one of the two complex PGDG were utilized upon encystment. Both of these components, however, regained almost their original levels after 5 hr of growth. The other PGDG and the MGDG decreased by about half upon encystment but increased greatly in the growth phase. Finally, the phospholipids PS, PI, and the peak containing PE and LPE decreased upon encystment whereas PC and LPC increased. All labeled phospholipids increased after 5 hr of growth, with PE and LPE becoming the major constituents.

DISCUSSION

Zoospores of *B. emersonii* can swim in nutrient-free media for extended periods of time[6,8] during which lipid is utilized.[6] Supportive electron micrographs[6] of starving zoospores showed an apparent progressive decrease in the size and number of lipid bodies as well as a continuous decrease in the size of the "lipid sac matrix" (also called "SB-matrix" or simply "SB"),[3] an organelle of unknown function. Our preliminary studies of isolated SB-lipid complexes suggest that the SB matrix contains a large amount of MGDG. Therefore, since MGDG is partially depleted during swimming, its loss might have been responsible for the decrease in the size of the SB-matrix. The major lipid component utilized during 5 hr of swimming, however, is the TG; we suggest, therefore, that this is the major lipid constituent of the lipid globules.

After 5 hr of swimming the TG had leveled off; between 5 and 10 hr, it increased while phospholipids decreased sharply. The utilization of phospholipid as energy sources has been cited for zoospores of *Phytophthora capsici*,[9] mammalian spermatozoa,[10,11] and *Tetrahymena pyriformis*.[12] Interestingly, there was a transitory increase in cytoplasmic triglyceride globules during starvation of *Tetrahymena*; the authors felt that fatty acids released by catabolism of phospholipid, possibly coming from broken mitochondria, were temporarily stored in the form of triglycerides.

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During encystment of *B. emersonii*, major changes take place in the glycolipids. All glycolipid components decrease; one, a PGDG, is completely utilized. The main glycolipid, a DGDG, decreases by 55%. This glycolipid is a major lipid constituent of the gamma particle,[13] an organelle involved in cyst wall synthesis.[14,15] The neutral lipid class remains constant upon encystment, while the phospholipid increases, due mainly to PC and LPC.

After 5 hr of growth, glycolipids had exceeded their level in 0 hr spores, while the phospholipid increased, due primarily to LPE and PE. The neutral lipids also changed conspicuously during growth with both the SE and MG being synthesized. The MG are probably being used for the synthesis of more complex neutral lipids and possibly indirectly for the synthesis of glycolipid and phospholipid components.[16] The reason for the increase in SE is not known although similar increases have been reported to occur during sorocarp formation of *Dictyostelium discoideum*.[17] Answers to this and related questions will hopefully be forthcoming from current studies of changes in the lipid content of subcellular organelles during development of *B. emersonii* zoospores.

EXPERIMENTAL

Culture techniques. Blastocladiella emersonii was grown at 22° in 9 l. modified PYG broth.[18] The culture, inoculated with $2{\cdot}5 \times 10^8$–$4{\cdot}5 \times 10^8$ spores obtained from PYG agar plates, was aerated (10 l./min) and illuminated with "cool white" fluorescent lighting, 555–712 $\mu W/cm^2$. After 9 hr of growth 50 μCi of NaOAc-[U-^{14}C], (54 mCi/mM) and NaOAc were added (final concn, 5×10^{-4} M). Growth was continued for an additional 9 hr at which time aeration was stopped, the plants were allowed to settle, and the spent medium was removed by suction. The plants were washed once with 4 l. of sporulation inducing medium (0·5 mM MOPS (Calbiochem), pH 6·8, containing 0·1 mM $CaCl_2$) and resuspended in 1 l. of the same solution; aeration was resumed. From 10^{10} to $3{\cdot}5 \times 10^{10}$ spores were synchronously released 6–7 hr later.

Suspensions of zoospores were passed through filter paper and concentrated by centrifugation (0 hr spores) or they were treated in one of two ways: (a) the spores were allowed to swim in the aerated buffered medium for 5 to 10 hr or (b) they were induced to encyst by chilling to 4–6° (avg. time required to reach temp., 45 min), then immediately adding PYG broth to full strength and equilibrating to 22° (*ca.* 95% encystment occurred within 15 min, this being faster than in most non-nutrient systems[15]) and then extracted immediately or after 5 hr of growth (germlings with mainly two nuclei).

Lipid extraction. Zoospores, cysts and germlings were conc (1000 *g* for 7 min), washed with H_2O and then recentrifuged. The pellets were suspended in 2:1 $CHCl_3$–MeOH (19 vol. solvent/l. vol. material), sonicated at 80 W for 30 sec, and extracted overnight at room temp. The extract was filtered through a coarse fritted glass Büchner funnel, evaporated to dryness under N_2, and suspended in 5 ml $CHCl_3$–MeOH (19:1). Non-lipid contaminants were removed with Sephadex G-25.[19]

Chromatography. The lipid was then applied to a silicic acid column (10 *g* silicic acid, 2 × 8 cm column) and separated into classes. Neutral lipids were eluted with 100 ml $CHCl_3$, glycolipids with 100 ml Me_2CO and phospholipids with 100 ml MeOH. Each class was taken to dryness under a stream of N_2 at 40° and resuspended in 2 ml $CHCl_3$–MeOH (2:1). The lipid classes were then used for dry wt determination, TLC, and measurement of total ^{14}C incorporated. Neutral lipids were separated on Gelman ITLC-SG chromatography media, using light petrol–Et_2O–HOAc (80:20:1). Glycolipids and phospholipids were chromatographed on ITLC-SA and ITLC-SG media respectively, using *iso*-PrOH–NH_4OH (100:7). Lipids were located by using H_2SO_4 and heat and identified by comparison with previously identified *B. emersonii* spore lipids.[7] Quantitative data for individual components were determined from total peak areas obtained from scans (Tracerlab 4 Pi scanner) for radioactivity in uncharred TLC's.

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